IRREGULAR TYPHOID STRAINS AND THE INFECTIONS CAUSED BY THEM

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In a review recently written by V. C. Vaughan [1] on a report published from the Public Health Laboratories of Cairo, Egypt, entitled "The Bacteriologic Examination of Suspected Typhoids," the following statement is made:

> "It is possible and indeed highly probable that so far as vaccination has failed it is due to the disease being caused by other members of the typhoid group, which in all probability is much larger than we now appreciate. A second most interesting point brought out in this valuable report is that of the introduction of a disease into a country where it has not hitherto prevailed and the possibility of the newly introduced organisms supplanting kindred organisms already native to the country. There are many reasons for believing that in the various camps in this country different organisms became predominant and the dominating organisms changed from time to time with new importations. It is possible that the great differences in the death rates in the various camps in this country may have been due to this or similar causes."

The observations reported in this paper lend some support to the suggestion mentioned, namely, that the failures of antityphoid-paratyphoid vaccination may be the result of other members of the typhoid group or, as it appears in our case, to be variants of typical B. typhosus. Minor variations among strains of the same species of bacteria are not uncommon, but in rare instances it has been possible to detect some of the factors that induce the production of such variants. With the creation of a large stratum of population highly protected by vaccination against typhoid and paratyphoid fever, the appearance of isolated cases or of small group epidemics in the supposedly immune must suggest an explanation similar to the one offered in the review. Unfortunately, the analysis of the various strains of B. typhosus isolated from vaccinated typhoid fever cases are few, the available data are unreliable and mostly obtained by incomplete biochemical and

Received for Publication March 16, 1920.

[1] Jour. Lab. & Clin. Med. 1919, 4, p. 645.

serologic methods. In fact, the recent findings of F. Mock [2] in 45 positive typhoid and paratyphoid fever cases at Mesves Hospital in France, representing cultures from vaccinated soldiers, emphasize this statement in every respect. It is not surprising to find in his paper, dealing with variants and irregular strains of the typhoid and paratyphoid group of bacteria, the conclusion that "these atypical paratyphoid organisms probably are involution forms of the true typhoid and paratyphoid bacteria." Some of his strains changed their cultural while others exhibited irregularities of their agglutination characteristics. It is therefore not unlikely that a more careful analysis of these strains would have forced a modification of the sweeping conclusions and would have led to a consideration of the epidemiologic importance of such atypical strains.

A review of the literature indicates that irregular typhoid and paratyphoid strains have been repeatedly reported.[3] Recent publications also call attention to the existence of nongas-producing paratyphoid and B. enteritidis strains, which may be readily mistaken for true B. typhosus, when only abbreviated methods for identification are employed.[4] In this connection we recall the conclusions of Tenbroeck [5] in his paper on a nongas-producing hog cholera bacillus in which the statement is made that his strain resembles in many respects B. typhosus, and it may be that some of the so-called typhoid cultures that are not agglutinated by antityphoid serum are nongas-producing paratyphoids. A detailed consideration of the other publications will be given in connection with the discussion of our own results.

Even since 1913, when one of us (K. F. M.)[6] analyzed rather superficially an irregular strain of B. typhosus, which had been isolated from a vaccinated army officer, we have studied several hundred typhoid and paratyphoid cultures isolated from man or after prolonged sojourn in the tissues of laboratory animals. Only recently two irreg-

[2] Jour. Lab. & Clin. Med. 1919, 5, p. 54.

[3] See the publications of: LeCount and Kirby: Trans. Chicago Path. Soc., 1903-1904, 6, p. 209; Faroy: Compt. rend. Soc. de biol., 1908, 64, p. 1093; Lafforgue. ibid., 1908, 65, p. 109; Marotte: Progrès méd., 1909, No. 28, p. 358; Babes and Feodorasco: Compt. rend. Soc. de biol., 1909, 66, p. 787; Fromme: Centralbl. f. Bakteriol., 1911, 58, p. 445; Goebel: ibid., 1914-15, 75, p. 376; Niolle, Raphael et Debains: An. de l'Inst. Pasteur, 1917, 31, pp. 373, 388 and 403; Raynaud and Négre: Compt. rend. Soc. de biol., 1912, 72, p. 534; MacAdam: Jour. Roy. Army Med. Corps, 1919, 33, p. 140.

[4] Messerschmidt: Centralbl. f. Bakteriol., 1912, 66, p. 35; Oette: ibid., 1913, 68, p. 1; Wagner: Ibid., 1913, 71, p. 25; Ohno: ibid., 1915, 75, p. 288; and Morse and Tyron: Boston Med. & Surg. Jour., 1917, 177, pp. 173, 216 and 255; Broughton-Alcock: Lancet, 1919, 2, p. 1023.

[5] Jour. Exper. Med. 1916, 24, p. 213.

[6] See footnote 2 in Riesman: Jour. Am. Med. Assn., 1913, 61, p. 2205.

ular typhoid strains isolated from two vaccinated laboratory workers attracted our attention. The epidemiologic circumstances leading to the infection and the interpretation which our observations suggest justify in our opinion a detailed account of the clinical and bacteriologic observations. The evidence to be presented also indicates that one of the irregular strains reverted to its typical ancestor by passing through an aged nonimmunized man. The history of the infections and the bacterial findings are:

CASE 1.—A janitor in our animal house, aged 59, complained of chilly sensations, weakness and severe frontal headache on April 1, 1919. He had a temperature of 38.8 C. and was therefore admitted to the hospital. There was nothing noteworthy in his family history. About March 10, 1919, he had felt chilly and feverish for two or three evenings in succession, but in the mornings he was always able to attend to his duties. However, during the day he perspired freely and was more readily fatigued in carrying his usual load of feed and distilled water, etc., upstairs. On account of the severe illness and the death of his wife he had very little rest during the months of January and February. In July, 1917, he was given three injections of fresh army T. A. B. vaccine; each injection was followed by a moderate local reaction. He has had for the last two years intimate contact with rabbits, guinea-pigs, cats, dogs and goats that discharged living typhoid bacilli. The two months preceding his illness about 30 to 40 rabbits, either renal or gall-bladder typhoid bacilli carriers, and numerous guinea-pigs infected spontaneously with rodent paratyphoid B. bacilli and B. enteritidis were kept in the section of the animal house under his supervision. The same caretaker handles also the cremation of these animals, which are always carefully wrapped by the experimenter in heavy paper. He never took any meals in the animal house and used water, soap and cresol solutions before returning to his home. However, in his spare moments he smoked cigarets, which he rolled himself.

Physical Examination.—The patient was well nourished and well developed; aside from his flushed cheeks he did not look very ill. There were no rose spots and no glandular enlargements. Liver and spleen were not felt. Both lungs showed normal dulness and breath sounds. The urine showed no albumin, sugar or sediment; the diazo reaction was negative. The blood count was 4,300,000 and 7,200 with 65% neutrophils and 24% lymphocytes. Temperature was 38.6 degrees, pulse 90, and respiration 26.

From the blood cultures (5 c c of blood in 200 c c of glucose broth and 2 c c in 10 c c glycerin peptone ox bile) (taken in the afternoon of April 1, 1919) a gram-negative typhoid-like organism grew in 18 hours. The agglutination test made on the same day was:

B. typhosus formalinized antigen, 1:10, + + +

B. typhosus living polyvalent, 1:40, + +.

B. typhosus paratyphosus A, formalinized, 0.

B. typhosus paratyphosus B, formalinized, 0.

On the third day of the patient's stay in the hospital, during which time the temperature did not rise over 38.2 C., it fell to 37 C. and remained normal. On April 5 and 9 he received intravenously 20,000,000 each of a polyvalent mixture of several strains of B. typhosus in addition to his own organisms.

The provocative injections produced a very slight hyperleukocytosis, blood cultures taken immediately after the clinical reaction remained sterile. Stool and urine cultures taken daily for 14 days failed to demonstrate organisms that could be identified with those isolated from the blood. The patient left the hospital on April 9 and returned to his work perfectly well on the 20th. On April 21 and 27 he received subcutaneously one billion each of the heatkilled, tricresolized bacteria, that is, the organism isolated from his blood. The agglutinations before and after the injections were as follows:

AGGLUTINATIONS BEFORE AND AFTER INJECTIONS OF BACTERIA

Date	Bacillus Isolated from Blood, Living	B. typhosus Formalinized	B. paratyphosus A Formalinized	B. paratyphosus B Formalinized
April 5	0	1:20 ++	0	0
April 8	0	1:20 ++	0	0
April 21	1:10 ++	1:20 +++, 1:40 ++	0	0
April 27	1:80 ++	1:40 +++	0	0
May 2	1:80 ++	1:40 +++	0	0

As several independent workers, by the use of the ordinary procedures, classified the bacillus isolated from the blood stream of the caretaker as a typhoid bacillus, the malady was also clinically diagnosed as a mild abortive form of typhoid fever in an aged and vaccinated man. Subsequent observations on the original milk and carbohydrate tubes and repeated agglutination tests threw doubt on the original identification and a more detailed study was contemplated as soon as other duties in the laboratory permitted. Such an inquiry became a necessity when another laboratory worker contracted an infection which was clinically diagnosed as typhoid fever and an organism similar to the one found in case 1 was isolated from the urine. The history of the second case is:

CASE 2.—A woman, aged 26, graduate student in bacteriology in this laboratory, complained of headache, general malaise, abdominal pains and remained absent from her work on June 2, 1919. For the last two months she had assisted in making thousands of agglutination tests of suspected typhoid colonies; in particular she had made several agglutination and fermentation tests with the bacillus isolated from case 1. In the course of these tests she examined also various plates that contained organisms of the B. enteritidis and B. paratyphosus B. rodent group. Her technic was clean and careful, and she always disinfected her hands thoroughly before leaving for meals. In July, 1918, she was vaccinated with T. A. B. Navy vaccine at Mare Island; each injection produced a moderately severe reaction. In October, 1919, she had a severe attack of pandemic influenza.

From June 2 until June 15, neither of us saw the patient, who lived out of the city, but a tentative diagnosis of abortive typhoid was suspected by the physician. To confirm this diagnosis we offered our services and con-

ducted repeatedly laboratory tests which are summarized in table 1. About June 20, the patient having been afebrile was permitted to leave the bed. She suffered a relapse on June 22 and was after that attended regularly by a physician and a nurse. The temperature chart available shows two typical enteric fever relapse curves, one extending from June 28 to July 12 and the other from July 14 to July 25. The only clinical data available state that the course was severe, accompanied in the last relapse by delirium. No rose spots were noticed, the spleen was never distinctly palpable but the pulse and blood count were characteristic for typhoid fever. Beginning June 28 she received a high calory carbohydrate diet. Her recovery was uneventful and complete. Our laboratory findings, which have a bearing on the problem to be discussed, are for the sake of clearness presented in tabulated form.

TABLE 1

LABORATORY FINDINGS IN CASE 2

Date	Leukocytes	Agglutination		Blood Cultures	Urine Cultures
		B. Typhosus	B. Para A, B, Dysenteriae, B. Melitensis		
June 15	7,300	1:20	0	Sterile	Negative
June 25	9,300	1:100	0	—	Negative
July 4	—	1:640	0	—	Positive, 6 colonies
Aug. 27	—	1:1000+	1:10-1:100	—	—

The facts stated, in conjunction with a consideration of the bacteriologic findings, are of considerable interest. A laboratory worker vaccinated against typhoid and paratyphoid developed a clinically typical typhoid fever infection. Agglutination and blood cultures failed to support this diagnosis until a bacillus identical in every respect to the one found in case 1 was isolated from the urine in the fifth week of the disease. Blood cultures were taken only in the second week, when the temperature was declining and the stool cultures were undoubtedly negative on account of the high calory carbohydrate diet, which had been given to the patient since June 10. The patient apparently was shedding organisms in the first two to three weeks' period of her illness as was indirectly demonstrated by the occurrence of another typical case of typhoid fever in her household. The history of this patient is:

CASE 3.—The father of patient in case 2, aged 70, unvaccinated, complained of malaise and headache on July 24. From that date until August 9 his temperature rose to 102 and 104 F., but he was not sufficiently ill to follow the advice of his physician which was to remain in bed. On August 9 agglutination was found positive by a board of health laboratory, and he was subsequently kept in bed. From the few data available it is evident that his typhoid infection was typical, very severe and ended fatally on Sept. 8, 1919. The clinical diagnosis was well supported by a few examinations we were able to conduct on the patient on Aug. 25, 26, and 29, 1919.

FINDINGS IN CASE 3

Date	Agglutination		Stool Culture	Urine Culture
	B. typhosus Formalinized	Case 1 Bacillus, Living		
Aug. 25	.....	.....	∞ typical B. typhosus	
Aug. 26	.....	.....	.....	∞ B. typhosus
Aug. 27	.....	.....	.....	300 million B. typhosus per c c of urine 4 hours after collection
Aug. 29	1:400 +++; 1:800 ++	1:600 +++; 1:800 ++	Almost pure B. typhosus	90 million B. typhosus per c c of urine 9 hours after collection

As Sch. never entered the room of the patient and the most scrupulous precautions in sterilizing all secretions had been taken by the nurse who attended case 2 since June 28, it was for a considerable period impossible to connect his infection with the one of his daughter, discussed in case 2. Sch. had remained in his home and our most searching epidemiologic inquiries failed to find an outside source where he could have contracted the disease. On further detailed analysis of the circumstances leading to the illness of her father, the daughter remembered that on or about June 20 when very ill she prepared unknown to her mother who attended her a specimen of her own stool and instead of sterilizing the applicator, threw it in the toilet. On July 12 a plumber was called to clean the clogged siphon. Sch. assisted him, removed and handled the applicator and commented to his wife and the nurse on the negligence of the person, who threw the piece of wood in the lavatory. Twelve days later on July 24 he noticed the initial symptoms of his typhoid infection.

The occurrence of this indirect contact case would in itself, as from an epidemiologic point of view, be of little value, but in correlation with the bacteriologic findings, the history can be appreciated and analyzed more carefully.

BACTERIOLOGIC IDENTIFICATION OF THE ORGANISMS ISOLATED FROM CASES 1, 2 AND 3

As stated in the histories, the bacillus isolated from the blood stream of case 1 behaved irregularly when tested by more detailed carbohydrate and serologic tests; the organism found in one urine sample of case 2 corresponded with the organism of case 1, with the exception of a marked hyperagglutinability in the first 16 transplants on digest or veal agar. The organism isolated from case 3 was easily identified as a typical typhoid bacillus. In the course of the epidemiologic analyses of the recorded findings it became necessary to compare the isolated organisms with the various typhoid strains to which the laboratory workers were suspected of having previously been exposed. It was, however, impossible to demonstrate conclusively the strain or strains of B. typhosus, which by passing through the body of case 1 had

become altered to an irregular typhoid bacillus, nor did we collect observations which could prove the infections were the result of an irregular B. enteritidis. Before discussing the various tests employed for the identification we state briefly the technical procedures used.

Blood cultures were made with from 5 to 10 c c of blood in glucose veal infusion broth (P_H 7.0) and peptone-glycerol-ox bile. Stool and urine specimens were plated on brilliant green-eosin-peptic digest agar. Urine samples were also enriched with an equal amount of peptic digest broth.

The isolated colonies of gram-negative nonlactose fermenting organisms were purified by repeated successive plating on peptic digest or veal agar. The three strains studied in this paper were also isolated by Burri's method as one cell cultures. The progenies of three cells of each strain were studied in peptone water-potassium phosphate-sodium chlorid-carbohydrate solutions with Andrade's or China blue rosolic acid indicator, Witte's peptone solution, bromcresol purple milk, neutral red, orcein and malachite green solution in 0.5% meat extract agar, and rhamnose-veal agar. The P_H^+ reaction of all mediums used was adjusted to 7.0-7.2. It will be shown in another paper that strain "I 75" of case 1 when first isolated was alkaline tolerant; the growth curve showed a marked plateau extending from P_H^+ 6.8 to 7.8. All tests reported have been repeated at least three times and the findings with a few minor exceptions to be discussed in detail remained constant. The parasitic strains differed in no way biochemically from the saprophytic ones; however, this statement cannot be applied to some of the serologic findings on the saprophytized offsprings of the two strains "I 75" and "Chr. 76." It appears advisable for the sake of clearness to discuss the various characteristics under separate headings.

Morphology.—The three strains "I 75," "Chr. 76" and No. 49 are morphologically indistinguishable from the typhoid type strain "Rawlings"; they are gram-negative and actively motile. They show differences in size during their growth on mediums identical with those described by Clark and Ruehl;[7] on very alkaline mediums filamentous rods are frequently noted.

Surface Colonies.—On dye mediums or on plain agar the parasitic strain of "I 75" and "Chr. 76" produced vine leaf shaped granular colonies. As a rule the colonies were always somewhat larger and the characteristic growth permitted recognition of the irregular strains in a mixture with a typical B. typhosus. The inside structure shows a rather fine striated network of furrows, which are readily visible with the naked eye. Indeed the colonies correspond in many respects with those recently described by v. Lingelsheim and Sachs-Müke[8] as so-called Q-strains. Recent tests with the more saprophytic strains produced irregular grayish or slightly yellowish lobulated colonies, which developed raised centers and some isolated colonies may occasionally show indications of slimy edges. These changes occur only when the plates after incubation for 18 hours at 37 C. are kept at room temperature and again the mucous appearance of the edges is only slight in comparison with those constantly noted on typical paratyphoid B. strains. On gelatin plates the typical leaf-like appearance of the colonies is more pronounced than on the agar plates, the medium is never liquefied.

[7] Jour. Bacteriol., 1919, 4, p. 615.
[8] Centralbl. f. Bakteriol., 1913, 68, pp. 577 and 582.

Lead Acetate Reaction.—The medium prepared according to Jordan and Victorson[9] is slowly reduced without the production of gas; the hydrogensulfide reaction is identical with the one noted for typical typhoid strains tested simultaneously.

Carbohydrate Reactions.—Strain "I 75" and "Chr. 76" ferment without gas production the following carbohydrates: glucose, levulose, galactose, mannose, mannite, maltose, xylose, dextrin, arabinose (3 times of 5 tested), dulcite and rhamnose. Strain "49" failed to ferment arabinose, dulcite and rhamnose in the observation period of 30 days. It is generally stated in textbooks and emphasized by Winslow, Kligler and Rothberg[10] in their studies on the classification of the colon typhoid group, that the type strain "Rawlings" does not attack arabinose, dulcite or rhamnose. Recent studies by Teague and Morishima[11] confirming previous observations made by Penfold,[12] Wagner,[3] Ditthorn[13] and others indicate that at least 6% of their typhoid cultures showed acid production in arabinose and from 14 to 37% in dulcite broth, when the period of observation was extended to 30 days. Of the 14 typical typhoid strains, which are under suspicion of containing the strain responsible for the infection in case 1 and used by us for comparison, 2 or 14% fermented repeatedly arabinose on the 7th or 14th day and 6 or 48% acted on dulcite in the one test, in which all strains were tested simultaneously. Repeated tests of our strain "I 75" and "Chr. 76" in arabinose-peptone-indicator solution confirmed the observations of Teague and Morishima that the acid production in this carbohydrate is irregular. In an early series with the parasitic strains acid production was noted in from 4 to 6 days; in another series with the saprophytic strains the reaction was delayed for 15 and even 24 days. On the other hand, the fermentation of dulcite was fairly regular; as a rule, acid was formed in from 2 to 4 days; in one series of tests a delay of 8 days was recorded. In some tests with dulcite the indicator was slightly reduced.

On endoplates prepared with arabinose instead of lactose, strain "I 75" and "Chr. 76" produced inside of the large isolated colonies in from 5-7 days one or several bud-like daughter colonies. Transplants from the papillae fermented arabinose in 24 hours. These observations on fuchsin-arabinose-agar are in many respects similar to those described by one of us (K. F. M.) for the paracolon bacilli isolated from calfscour's.[14]

On dulcite-endoplates also red papillae are produced about the 8th to 10th day, but transplants from these behaved irregularly; an observation which we found confirmed by the recent publication of Teague and Morishima.

The fermentation of rhamnose or isodulcite is regularly noted in any liquid medium chosen. Sometimes 3-5 days elapse before distinct acid reaction is shown by the indicator; in some series the acid production was confined to the flocculent growth sediment of the tubes and only 3-4 days later the acidity diffused throughout the liquid. According to Krumwiede, Kohn and Valentine[15] and Winslow, Kligler and Rothberg,[10] who have recently tested a series of typhoid strains, it is generally believed that the B. typhosus does not ferment this particular carbohydrate and the bacillus can therefore readily be differ-

[9] Jour. Infect. Dis., 1917, 21, p. 554.
[10] Jour. Bacteriol., 1919, 4, p. 472.
[11] Jour. Infect. Dis., 1920, 26, p. 52.
[12] Jour. Hyg., 1912, 12, p. 195.
[13] Centralbl. f. Bakteriol., 1912-13, 67, p. 497.
[14] Jour. Infect. Dis., 1916, 19, p. 700.
[15] Jour. Med. Research, 1918, 38, p. 89.

entiated from the members of the paratyphoid group. Penfold,[16] on the other hand, states that "growth of B. typhosus on isodulcite broth frequently does not produce acidity though it may do so as early as one week." Thus it is quite evident that this pentose in a liquid substratum is not of much value for distinguishing irregular strains of B. typhosus from nongas-producing paratyphoid strains.

Rhamnose-Agar Papillae Reaction.—In this connection it should be recalled that R. Müller[17] and later Penfold,[16] Saisawa,[18] Wagner,[3] Teague and Morishima[21] consider the development of daughter colonies on rhamnose agar a specific reaction for typhoid bacilli. In a series of tests with a small amount of rhamnose available we were able to confirm this specificity. Strain 49 and 4 other typhoid strains, representatives of the three groups of Hooker's serologic classification produced immunerable dense papillae in from 48 to 72 hours. Strain "I 75" and "Chr. 76" in spite of vigorous growth developed only small daughter colonies about the 10th or 12th day of incubation; they rarely reached the size of those noted with the typical typhoid strains and always remained translucent. Strain "I 75" and "Chr. 76" evidently differ in the rhamnose-papillae reaction from the typical typhoids which in part explains their ability to ferment this carbohydrate by acid production. The freshly isolated, as well as the saprophytic, strains behave in an identical manner. Thus far no reversion to the true type has been observed.

Raffinose-Agar Papillae Reaction.—Neither of the three strains concerned in this publication produced papillae on raffinose agar. The animal strains of B. paratyphosus B and B. enteritidis to which the workers had been exposed, all showed centrally located daughter colonies.

Little need be said with regard to the fermentation of xylose. We fully agree with Teague and Morishima that the so-called xylose nonfermenters are in reality slow fermenters. Strain "I 75" and strain "Chr. 76" were rapid xylose fermenters and maintained this property when repeatedly tested during the last 8 months.

Bromcresol-Purple Milk.—Both strains "I 75" and "Chr. 76" are characterized by rapid alkaline production in milk. The initial slight acidity is changed on the 3rd to the 5th day to a decided alkalinity which progressively increases and leads to saponification of the milk fat on the 15th to 30th day. The rapidity with which the reaction changes from a $P_H{}^+$ 6.6 to $P_H{}^+$ 9.0 in this medium has somewhat slowed down in these cultures kept for 6 months on plain peptic digest agar. The two strains as second and third generations isolated from the human body produced a deep purple reaction ($P_H{}^+$ 8.6) inside of 5 days. Quite recently tested, at least 10 to 15 days elapsed until the same degree of alkalinity under the same conditions was noted. We have made similar observations on several strains of B. sanguinarum kept on agar for nearly 4 years; originally rapid they have gradually changed to slow alkali producers. It was noted in a series of tests with strain 49 that often at the end of 30 days' incubation about 50% of the inoculated milk tubes gave decided alkaline reactions. For example, transplants made from 12 isolated colonies on plain agar into milk tubes of the same lot, produced after varying intervals the following reactions:

[16] Brit. Med. Jour., 1910, 2, p. 1672.
[17] Centralbl. f. Bakteriol., 1911, 58, p. 97 and Münch. Med. Wchnschr., 1909, 49, p. 885.
[18] Ztschr. f. Hyg. u. Infektionskrankh., 1913, 74, p. 61.

REACTIONS OF TRANSPLANTS FROM TWELVE ISOLATED COLONIES ON PLAIN AGAR INTO MILK TUBES OF SAME LOT

	Milk Reaction		
	On 1st Day	On 16th Day	On 30th Day
1. Smooth colony	P_H.6.8	P_H+.6.2	6.2
2. Smooth colony	P_H.6.8	P_H+.6.2	6.2
3. Smooth colony	P_H.6.8	P_H+.7.7	7.8
4. Smooth colony	P_H.6.8	$P^+{}_H$.7.7	7.7
5. Smooth colony	P_H.6.8	P_H+.7.7	8.0
6. Smooth colony	P_H.6.8	P_H+.6.2	6.2
7. Smooth colony	P_H.6.8	P_H+.7.7	8.5
8. Smooth colony	P_H.6.8	P_H+.7.3	7.3
9. Vine leaf like colony	P_H.6.8	P_H+.6.8	6.8
10. Vine leaf like colony	P_H.6.8	P_H+.6.8	7.0
11. Lobulated colony	P_H.6.8	P_H+.7.5	7.7
12. Lobulated colony	P_H.6.8	P_H+.6.2	6.2
Strain "I 75." One vine leaf colony	P_H.6.8	P_H+.8.5	9.0 gelatinized

The differences in the final P_H+ reaction are merely the result of differences in the rate of multiplication of the selected colonies. Strain "I 75" in comparison with strain 49 possesses a more rapid growth rate, which in turn gives rise to alkaline split products in a correspondingly shorter time interval. It is as yet undecided, whether the alkaline reactions were caused primarily by the oxidation of the salts of citric acid to alkaline carbonates as recently suggested by Ayers, Rupp and Johnson[19] or the result of a true alkali fermentation. Some incomplete data at our disposal support strongly the contention of the workers mentioned.

Bradley,[20] Krumwiede, Pratt and Kohn[21] and recently Jordan[22] pointed out that the differences in the milk reaction of the various paratyphoid strains are probably due merely to a difference in the rate of multiplication. A similar mechanism seems operative in the milk reactions of the typical and the so-called "blue typhoids." Even different members of one and the same strain may develop in milk rapid or slow alkali-producing offsprings. Bromcresol purple milk can therefore not be recommended as a suitable differential medium for the classification of irregular strains.

Indol Production, Methyl Red Test and Hemolysis.—In one tube of Witte's peptone solution inoculated with the second generation of strain "I 75" a slight but definite indol reaction was noted with Ehrlich's reagent on the sixth day. Subsequent tests, including strain "Chr. 76," gave negative results. Through the observations of Andrejew[23] and Bull and Pritchett[24] it is known that irregular typhoid strains occasionally produce traces or even large amounts of indol. The methyl red test was positive. All 3 strains failed to produce hemolysis on blood-agar plates.

Reduction of Dyes.—Neutral red in 0.5% agar with or without glucose was not reduced by the 3 strains under discussion. A slight slow reduction (5 days) of malachite green and orcein agar was observed, but also constantly noted with the control typhoid strains.

[19] U. S. Depart. Agr. Bull., 782, 1919.
[20] Jour. and Proc. Roy. Soc. N. S. Wales, 1912, 46, p. 74.
[21] Jour. Med. Research, 1916, 35, p. 55.
[22] Jour. Infect. Dis., 1918, 22, p. 511.
[23] Arb. K. Gsndttsamte, 1910, 33, p. 363.
[24] Jour. Exper. Med., 1916, 24, p. 39.

The detailed findings may be briefly summarized: Biochemically, strain "I 75" and "Chr 76" differ from the true typhoid strains, including strain No. 49, by their intensified carbohydrate reactions; dulcite, rhamnose and arabinose broth in the order stated are acidified in a shorter time interval than is customarily recorded for B. typhosus. This accelerated ferment action may also be in part responsible for the rapid alkali production in milk, which we found apparently was the result of a more rapid and more intense growth in this medium than is ordinarily observed with typical typhoid strains. Both strains fail to produce papillae on raffinose agar, but do so on rhamnose plates.

SEROLOGIC IDENTIFICATION

A suspension of the gram-negative, motile bacilli, which grew in the blood culture of case 1, was promptly clumped by a polyvalent typhoid immune serum in a slide and in a macroscopic agglutination test. Additional determinations made with specific rabbit antiserums and suspensions of living bacteria of strain "I 75" placed this organism serologically with the typhoid bacillus. The reactions were always well marked with typhoid serum and coreactions with other serums were slight or absent. In the course of several months, when an attempt was made to determine more closely the group relationship of strain "I 75" it was found that this organism when preserved in a 0.1% formalinized salt solution was inagglutinable by B. typhosus serum, but had apparently acquired the property of being specifically clumped by B. enteritidis serums in maximum dilutions. This striking specificity of the killed in contrast to the living suspensions has been occasionally noted with other strains, but never to such a degree as was constantly encountered in the tests with strain "I 75." In the literature we found only the statements by Kafka,[25] Klemens,[26] Minelli[27] and others, that formalinized suspensions may show complete absence or marked reduction of coagglutination reactions. On the other hand, most writers agree that agglutination reactions with living bacteria must be considered more sensitive than those conducted with dead cultures. It is, however, evident that little attention has been paid to this phenomenon and a careful study of immunologic and physicochemical factors responsible for this differences suggest themselves. Repeated tests conducted during the last 8 months with at least 20 different formalinized and living suspensions always gave identical and uniform results, which are shown in table 3.

Strain "Chr. 76" was hyperagglutinable when first isolated and could only be tested after 30 successive transplantations on neutral peptic digest agar. By changing the electrolyte contents of the suspensions and serum dilutions, in using a 0.25% salt solution according to the method of Verzàr[28] specific reactions and slight coreactions occurred with typhoid serum and living suspensions. These preliminary tests placed, in our opinion, strain "Chr. 76" with the typhoid bacillus. Several months after the date of isolation when absorption tests were in the process of preparation agglutination tests with formalin-

[25] Centralbl. f. Bakteriol., 1906, 40, p. 247.
[26] Berl. klin. Wchnschr., 1905, 42, p. 1269.
[27] Centralbl. f. Bakteriol., 1906, 41, p. 583.
[28] Centralbl. f. Bakteriol., 1917, 80, p. 161.

ized suspensions were undertaken. Again the same phenomenon, as already described for strain "I 75," became apparent, namely strain "Chr. 76" was inagglutinable in formalinized killed suspensions; it was, however, specifically

TABLE 3

AGGLUTINATION REACTIONS

Antiserums	Antigen "I 75"		Antigen "Chr. 76"		Antigen "49"	
	Living Second Generation	Formalinized 0.1% 21st Generation	(Living Second Hyperagglutinable) 15th Generation in 0.25% Saline Specific Agglution	Formalinized 0.1% 30th Generation	Living Second Generation	Formalinized 0.1%
Polyvalent B. typhosus (1:40,000)	1:600 (blood broth) (bile broth)	0	1:1000+++	0	1:20,000	1:40,000
Hooker's Group I — B. typhosus "9" (1:10,000)	1:2000 (20th gener.)	0	1:1000+++	0	1:10,000 (10th gener.)	1:10,000
Hooker's Group I — B. typhosus "11" (1:10,000)	1:200+++ (20th gener.)	0	.....	.....	1:10,000 (10th gener.)	>1:10,000
Hooker's Group II — B. typhosus "Rawlings" (1:8,000)	1:600+++ (20th gener.)	0	1:600++	0	1:8,000 (10th gener.)	1:6,000
Hooker's Group II — B. typhosus "Dorset" (1:20,000)	1:400+++ (20th gener.)	0	.....	.....	1:20.000 (10th gener.)	1:20,000
Hooker's Group III — B. typhosus "Hopkins" (1:10,000+++)	1:600+++ (20th gener.)	0	1:600+++	0	1:10,000 (10th gener.)	>1:10,000
Hooker's Group III — B. typhosus "1" (1:6,000+++)	1:200 (20th gener.)	0	1:400+++	0	1:6,000 (10th gener.)	1:8,000
B. paratyphosus B polyvalent (1:20,000)	1:20+++	0	1:80++	0	<1:200	0
B. paratyphosus B human (1:10,000)	.....	0	.....	.....	<1:100	0
B. paratyphosus B avian (1:20,000)	.....	1:50±	.....	.....	.....	0
B. paratyphosus B rodent (1:20,000)	.....	0	.....	.....	.....	0
B. paratyphosus A (1:40,000)	1:40++; 1:60+	0	1:800+++	0	<1:100	0
B. sanguinarium (1:1,000)	1:200	0	1:100++	0	1:200	1:100
B. pullorum (1:2,000)	1:100	0				
B. enteritidis human III (1:20.000)	.....	1:2,000	.....	1:2,000	<1:100	0
B. enteritidis rodent I (1:10,000)	<1:50	1:2,000	.....	1:2,000	.....	0
B. enteritidis calf. (1:10,000)	.....	1:2,000	.....	1:2,000	.....	0
Normal rabbit serum	1:10	0	1:120	0	<1:10	0

agglutinated by B. enteritidis-serums. Strain "Chr. 76" in living suspensions is somewhat more readily sedimented and clumped by typhoid and paratyphoid serums than strain "I 75," but from the standpoint of the serologic data presented in table 4 the two strains must be considered as identical. The parasitic strains differ from the saprophytic ones by their ability of being readily agglutinated by B. enteritidis serums. Strain No. 49 behaves serologically like a

typhoid bacillus. No changes in agglutinability have been noted during the last 4 months. Standardized suspensions are specifically agglutinated by typhoid serums and group reactions are only noted with B. sanguinarum-serums.

The fact that formalinized killed suspensions of strain "I 75" and "Chr. 76" were not agglutinated by typhoid serums made the original diagnosis rather questionable and it was thought possible to determine the exact position of the bacteria under consideration by the use of a specific serum prepared with strain "I 75." On account of the high toxicity of this strain we succeeded only after many attempts in producing a highly specific and potent serum of a titer of 1:200,000.

TABLE 4

TESTS WITH STRAIN "I 75" ANTISERUM

Antiserum for Strain "I 75"	Living Suspended 0.1% Formalinized Salt Solution	Killed in 0.1% Formalinized Salt Solution
Strain "I 75"	1:200,000	1:20,000
Strain "Chr. 76"	1:100,000	1:80,000
Strain "Sch. 49"	1:1,000+++; 1:2,000++	0
B. typhosus "Rusk"	1:4,000+++	0
B. typhosus "Blair"	1:10,000++	—
B. typhosus "Singleton"	1:6,000+++	0
B. typhosus "Jacobs"	1:200+++	—
B. typhosus "Blunt"	1:1,000+++	—
B. typhosus "Moffitt"	1:200+++	—
B. typhosus "Kleeberg"	1:6,000+++	—
B. typhosus "Houston"	1:1,000+++	—
B. typhosus "Cordona"	1:8,000+++	—
B. typhosus "15"	1:4,000+++	—
B. typhosus "Kearney"	1:4,000+++	0
Hooker's Group I: B. typhosus "52"	1:200++	0
Hooker's Group I: B. typhosus "40"	1:2,000+++	0
Hooker's Group I: B. typhosus "11"	1:200	0
Hooker's Group II: B. typhosus "Dorset"	1:2,000+++; 1:4,000++	0
Hooker's Group II: B. typhosus "Rawlings"	1:6,000+++; 1:8,000++	0
Hooker's Group III: B. typhosus "1"	1:2,000+++; 1:10,000++	0
Hooker's Group III: B. typhosus "3"	1:4,000+++; 1:6,000+	0
B. paratyphosus "Human 26"	1:200+++	0
B. paratyphosus A "13, 15, 16"	1:400+++; 1:1,000+	0
B. sanguinarium "5"	1:100+++; 1:4,000++	0
B. enteritidis, strain 1, origin "rat"	1:200,000	1:20,000+++
B. enteritidis, strain 2, origin "A. M. N. H., unknown"	1:200,000	1:20,000+++
B. enteritidis, strain 3, origin "Strassburg, human"	1:200,000	1:10,000+++
B. enteritidis, strain 6	>1:200,000	1:40,000+++
B. enteritidis, strain 13, origin "Calfscours"	1:200,000	1:20,000+++

It is clearly indicated that such a serum gave with living suspensions of a variety of typhoid and also paratyphoid A bacilli, pronounced and fairly uniform coreactions. On the other hand, the "I 75" antiserum agglutinated in formalinized suspensions only its own organism, strain "Chr. 76" and a number of B. enteritidis strains isolated from various sources. The coreactions obtained with the 11 typhoid strains, which are under suspicion of having been the sources for the infection of case 1, and the creation of strain "I 75"

were not sufficiently striking to stigmatize any particular one as being antigenically closely related to strain "I 75," and again, the strains which represent the 3 groups of Hooker's classification[29] are not influenced serologically by the "I 75" immune serum in such degrees that a relationship of our strain "I 75" to either one of these groups could be arbitrarily deducted. To be sure, the reactions appear more as group reactions which apparently embrace the entire typhoid-paratyphoid group.

At this stage of the serologic identification it was considered necessary to apply absorption tests, naturally using living suspensions as antigens, and absorbing the immune serum completely of their agglutinin content. Extensive experimental series with the organisms of the B. paratyphosus and B. melitensis groups have convinced us that only the complete removal of all immune substances will give comparable results. With highly potent serums, such as the one prepared with "I 75," the procedure of removal is very tedious; 4 to 8 saturations with living organisms are sometimes necessary to deprive the serum of its entire agglutinin content for the absorbing antigen. The technic used by us is similar to the one described by Taylor, a detailed account therefore appears superflous.

TABLE 5

ANTISERUMS AGGLUTINATE WITH LIVING ANTIGENS AFTER COMPLETE ABSORPTION

Strains	"I 75" with "I 75"	"I 75" with B. typhosus "9"	"I 75" with "Rawlings"	"I 75" with B. typhosus "3"	B. typhosus "Rawlings" with "I 75"	B. typhosus "3" with "I 75"	B. typhosus "Rawlings" with B. typhosus "3"
I "75"	0	1:200,000	1:200,000	1:200,000	0	0	0
Chr. "76"	0	1:100.000	1:100,000	1:100,000	0	1:40±	0
No. 49	0	1:40±	0	1:320	1:10,000	>1:5,000	1:40
Hooker's Group I							
B. typhosus "9"	0	0	0	1:40+	1:4,000	1:2,000	1:80
B. typhosus "11"	0	0	0	1:80	1:4,000	1:600	1:200
Hooker's Group II							
B. typhosus "Rawlings"	0	1:40±	0	1:320	1:10,000	1:2,000	1:600
B. typhosus "Dorset"	0	1:320	0	1:160	1:2,000	1:2,000	1:40
Hooker's Group III							
B. typhosus "Hopkins"	0	1:160	1:40	1:320	1:4,000	1:4,000	1:80±
B. typhosus "1"	0	1:320	1:640	1:640	>1:2,000	>1:5,000	0
B. typhosus "3"	0	.....	1:320	0	1:160	1:2,000	0

0 indicates agglutination less than 1:40.

Strain "I 75" removes from its own immune serum all coagglutinins for the B. typhosus. On the other hand, a typical B. typhosus recently isolated and belonging to group I of Hooker's classifications removes from the serum of strain "I 75" the immune substances for his own strain and closely allied representatives of group II and III. The agglutinins for strain "I 75" remain quantitatively intact. A similar phenomenon takes place when this serum is absorbed with representatives of groups II and III. It is, however, apparent that a B. typhosus strain belonging to group III deprives the immune serum "I 75" in repeated tests incompletely of its agglutinins for the representatives of groups I, II and III. From the studies of Hooker the rather heterogeneous composition of this group is known and irregular reactions actually characterize this subgroup of typhoid bacilli. In a typhoid immune serum prepared with

[29] Jour. Immunol., 1917, 2, p. 1.

the type strain "Rawlings" group II the strain "I 75" removes its own group agglutinins, but the major typhoid agglutinin remains practically unaltered. The effect of "I 75" on an immune serum prepared with an organism of group III B. typhosus 3 is identical, and again, a B. typhosus of group III removes from a group II serum not only the agglutinins for his own group, but also those of strain "I 75" and group I simultaneously, thereby reducing the active substances for group II. These preliminary absorption tests will be enhanced along various other, particularly quantitative, lines as suggested in the recent publication by Andrewes and Inman,[30] but they are, so far as it concerns typhoid serums, sufficiently definite to draw certain deductions. Speaking in terms of agglutinin content of these serums, it is evident that a strain "I 75" serum contains, aside from its own major agglutinin, coagglutinins for groups I, II and III of Hooker's classification and absorption with representatives of these groups removes only these group-agglutinins. A "Rawlings" or a group III serum, on the other hand, has group agglutinins for "I 75" which can be specifically absorbed by this strain. Agglutinins for group III in a group II serum remove also the immune substances for strain "I 75."

Serologically strain "I 75" and "Chr. 76" belong to the typhoid group of bacteria; they differ antigenically from the three groups of Hooker; but are closely related to his heterogeneous group III. We are unfortunately not in possession of the typhoid strains used by Weiss[31] and therefore cannot state in which antigenic subgroup mentioned in his study strain "I 75" and "Chr. 76" should be placed. One point is certain: Our strains stand apart as a definitely differentiable type, even when using living cultures. Moreover, their relation to the typhoid group was shown only by the use of living suspension; formalinized antigens were either inagglutinable or highly specific.

Attention has already been called to the interesting fact that strain "I 75" and "Chr. 76" gradually acquired the ability to be agglutinated in living, and in killed suspensions as well, by B. enteritidis serums and vice versa. This group of organisms was uniformly agglutinated to the titer limit by the specific "I 75" immune serums. On the other hand, strain 49 repeatedly tested was not agglutinated by any of the available B. enteritidis serum. At first the observation was explained by the well-known fact that B. enteritidis serums and vice versa B. typhosus serums in many instances give striking coreactions. Already Durham[32] and later Kutscher and Meinecke,[33] Liefmann[34] and others called attention to this peculiar serologic relationship of certain B. enteritidis strains to B. typhosus. Absorption tests, however, separated the two organisms in a decisive manner. Thus it would appear to be a simple procedure to determine whether our strains are true B. typhosus or true B. enteritidis. Our absorptions test produced, however, paradoxical results (see table 3).

Complete removal of the B. enteritidis agglutinin in a "I 75" immune serum deprives this serum also of the same substances for strain "I 75" and again a B. enteritidis serum absorbed with the irregular strain "I 75" or "Chr. 76" fails to agglutinate all of the B. enteritidis strains tested. Judging from these paradoxical results, which were repeated with various other completely and incompletely absorbed serums, we should conclude that strain "I 75" and "Chr. 76" are typical nongas-producing B. enteritidis strains. We searched in vain for similar observations in the literature, but could only find the references

[30] Medical Research Committee, Special Report, Series, No. 42, 1919.
[31] Jour. Med. Research, 1917, 31, p. 135.
[32] Lancet, 1898, 1, p. 154 and Brit. Med. Jour., 1898, 2, p. 588.
[33] Ztschr. f. Hyg. u. Infektionskrankh., 1906, 52, p. 30.
[34] München. med. Wchnschr., 1908, 55, p. 159.

already mentioned. Christiansen,[35] the only writer who is thoroughly familiar with the nongas-producing B. enteritidis or paracolon strains, failed to conduct absorption tests probably because his serum coagglutinated typhoid bacilli in dilutions, which did not suggest such procedures. Until extensive studies with a large number of B. typhosus and B. enteritidis strains have demonstrated the antigenic relationship of these bacteria, we only record our observations and abstain for the present from offering an explanation. It is not unlikely that the B. enteritidis coreaction is characteristic for atypical typhoid strains and in this respect may have considerable diagnostic value and may even strengthen our conception of the typhoid nature of strain "I 75" and "Chr. 76."

In this connection attention is directed to the observations of Sobernheim and Seligmann,[36] which indicate a peculiarly marked lability of the antigenic properties of many B. enteritidis strains. Two old laboratory strains of this organism showed a transformation of their biologic properties, which was frequently combined with changes in the cultural characteristic. Careful plating methods demonstrated a number of daughter colonies, which apparently represented the transitional stages between the original and the finally transformed irregular strains. These observations are suggestive when we recall that our atypical strains acquired agglutinability for B. enteritidis serums in the course of a saprophytic life on agar slants. Neither the biochemical functions of our strains, nor the susceptibility for specific agglutination with typhoid serums have, however, changed in the course of at least 150 transplants. This and similar observations have convinced us that all future publications on pathogenic micro-organisms should definitely state whether the biologic and biochemical studies recorded were made on parasitic or saprophytic offsprings of the original culture.

Identification by Pathogenicity and Protection Experiments.—The freshly isolated strain "I 75" was exceedingly toxic for rabbits; the symptoms and anatomic findings differed in no respect from those commonly seen in animals intoxicated by true typhoid bacilli.

Guinea-pigs of 250-300 gm. of weight succumbed to intraperitoneal inoculations of from 60-100 million living organisms. Careful immunization with heat-killed organisms even by subcutaneous application of the inoculum is difficult; about 50% of the guinea-pigs show progressive emaciation without organic changes or lesions commonly noted in paratyphoid infections. Rats fed for one entire week with broth culture of "I 75" eliminated the fed bacteria, but remained clinically well.

Protection Experiments.—Recent studies conducted in this laboratory and to be published elsewhere demonstrated that the tissues of typhoid immune and nonimmunized rabbits destroy in a given time interval (24-48 hours) approximately the same number of intravenously inoculated typhoid bacilli. On the other hand, paratyphoid immune rabbits can apparently dispose of an infection produced by an intravenous inoculation of paratyphoid organism more rapidly and more completely than the nonimmune animals. This principle was applied to the identification of strain "I 75."

Exper. 1:—On Dec. 16 rabbit 1, which had been intensively immunized with dead and living B. typhosus "Rawlings" (agglutination titer of the serum 1: 6000), rabbit 2, immunized in an identical manner with the strain "I 75" (agglutination titer of the serum 1: 100,000) and a normal rabbit of the same litter and weight (agglutination titer <1:10) were inoculated intravenously with 1 c c each containing 6.400 million living orgnisms of strain "I 75." On

[35] Centralbl. f. Bakteriol., 1914, 74, p. 474.

[36] Deutsch. med. Wchnschr., 1910, 36, p. 351.

Dec. 17, seventy-four hours after the injection of the infective dose, rabbit 3 was profoundly intoxicated and showed rapid breathing and diarrhea. The two immune animals appeared less active and ate little. All three rabbits were exsanguinated under ether; the organs were removed aseptically and portions of the same were pulped with sand and saline in sterile mortars and diluted in such proportions, that each cubic centimeter of saline contained 100 mg. of tissue pulp. This material was plated as dilutions in peptic digest agar. The plates were counted after 48 hours' incubation at 37 C. Table 6 illustrates the average number of viable bacteria demonstrated in the tissues and in the blood stream.

TABLE 6

EXPERIMENT I: INTRAVENOUS INJECTION OF 6,400 MILLION ORGANISMS. SACRIFICED AND TISSUES PLATED 24 HOURS AFTER INJECTION

Tissues	Rabbit 1 Immune to B. typhosus "Rawlings"	Rabbit 2 Immune to Strain I 75	Rabbit 3 Normal
Agglutination titer	1:6,000+++	1:100,000+++	<1:10
	Per 100 mgm. of tissue. The following Grew After 24 Hours' Incubation	Per 100 mgm. of tissue. The following Grew After 24 Hours' Incubation	Per 100 mgm. of tissue. The following Grew After 24 Hours' Incubation
Liver, left and center lobe	12,000	20,000	480,000
Liver, right and center lobe	30,000	15,000	720,000
Bile	3 per 0.8 c c	0	238,000,000 per 1.7 c c
Gallbladder wall	90	0	18,000
Spleen	380,000	110.000	6.300,000
Bonemarrow	13,200	70,000	1,680,000
Mesenteric lymphnodes	180	400	12,400
Kidneys	72	6,700	34.000
Lungs	17,500	1,000	640,000
Heart blood	.....	2,400	52,000
Carotis blood	200	.....	48,000
Duodenum	Negative for B. I 75	1 colony of B. I 75	20 colonies of I 75
Ileum	150 colonies per loopful of intestinal content	2 colonies of B. I 75	

It is quite evident that the normal animal is less readily capable of destroying the intravenously inoculated bacteria of strain "I 75" than the immune one. The profound intoxication is indicated by a high bacterial count of the bone marrow, an observation which has recently been emphasized by J. T. Parker. The bile and spleen are also heavily infected. On the other hand, there is little difference between the animal immunized against the infecting strain and the one protected against the "Rawlings" organism. Both rabbits are destroying the inoculated organisms in the chosen time interval of 24 hours in approximately equal proportions. From our extensive experience with this particular method of immunity research already referred to we are justified in concluding that strain "I 75" behaves in the immune and normal rabbit like a paratyphoid organism, but that apparently no differences exist between the destructive forces of the animal immune to the infective strain "I 75" and the one which is only protected against the type typhoid strain "Rawlings." This experiment again supports the contention that strain "I 75" is antigenically closely related to the typhoid bacillus.

Bull and Pritchett[24] and recently J. T. Parker[37] have emphasized the fact that rabbits immunized with typhoid bacilli are highly and specifically resistant to intoxication with this organism. They withstand, as a rule, from 30 to 40 lethal doses of the living bacilli. Unfortunately no experimental data are available which prove conclusively that the toxic substances derived from organisms of the typhoid-paratyphoid group are strictly specific and our tests along these lines have not sufficiently matured to enable us to express a final

[37] Jour. Med. Research, 1919, 39, p. 301.

opinion. It is therefore with some hesitancy that we record some protection tests, which in themselves are very suggestive and which should encourage further inquiry along these lines.

Exper. 2:—One rabbit immune strain "I 75." 2 rabbits to different strains of typical B. typhosus, 1 rabbit to B. paratyphosus B, 1 to B. coli and 2 controls were injected with 50 lethal doses of strains "I 75." The animals succumbed after the following time intervals:

TIME IN WHICH ANIMALS SUCCUMBED TO LETHAL DOSES

	Died
Normal rabbit	3 hours, 50 minutes after the injection
Normal rabbit	4 hours, 10 minutes after the injection
Immune to B. paratyphosus	5 hours, 5 minutes after the injection
Immune to B. coli	5 hours, 25 minutes after the injection
Immune to B. typhosus polyvalent	23 hours after the injection
Immune to B. typhosus polyvalent	26 hours ofter the injection
Immune to Strain "I 75"	36 hours after the injection

It is a known fact, that normal rabbits vary considerably in their resistance to bacterial toxins of the typhoid group. This in part explains the unfortunate use of an intoxicating dose which also proved fatal in the specifically immune rabbit. The results could therefore be made more definite, but at least they indicate, as presented, that true typhoid bacilli protect to a certain degree against strain "I 75" and that paratyphoid and colon immune rabbits succumb to the intoxication as readily as the nonimmune ones.

We also attempted in a series of immune and normal guinea-pigs to determine the distribution and destruction of strain "I 75." Thus far we have noted exceedingly interesting paradoxical results, namely, the specifically and the typhoid immune guinea-pigs succumbed to the infection in contradistinction to the normal animals which remained alive. Until we have sufficiently often repeated these observations and can offer an explanation for this phenomenon we are withholding for the present the citation of a detailed experiment.

DISCUSSION

Before entering into a discussion of the various problems that suggest themselves in the analysis of the data, it is necessary to present a summary of the events as they appear in our interpretation.

An animal caretaker, who had intimate contact with secreta and cadavers of typhoid experimental animals, contracted an abortive attack of typhoidal fever. The short duration of his illness is probably due to his age immunity and to the prophylactic vaccination which had been administered to him, one and one half years previous to his infection. On only one occasion was an irregular typhoid strain isolated from his blood stream. His Widal reaction and stool and urine cultures were always negative. About two months later a vaccinated laboratory worker, who was experimenting with the irregular strain and also with the cultures of B. typhosus used for our experimental work on animals, developed a severe attack of typhoid fever. An irregular strain of B. typhosus identical with the strain isolated from

the caretaker was demonstrated on one occasion in her urine. Repeated blood, stool and urine culture during the relapse periods were negative. Her unvaccinated aged father contracted typhoid fever twelve days after handling a wooden spatulum, which had been used by the patient to prepare her stool specimen for shipment to laboratory. The stool and urine of this case contained typical typhoid bacilli. From the standpoint of the bacteriologist the variants of the B. typhosus may have originated in the following manner: The highly immune caretaker atavistically changed one or several typical strains of B. typhosus to the irregular strain described as "I 75," which in time caused a second severe infection in a vaccinated young woman. Her strain in passing through the tissues of an old, nonimmunized man reverted to a typical B. typhosus.

The observations recorded in the foregoing paragraphs deserve, however, a more detailed consideration from three different points of view, namely: (1) epidemiologic, (2) clinical and (3) bacteriologic.

1. *Epidemiology.*—As already outlined in the history of case 1, we feel convinced on epidemiologic grounds that our animal caretaker contracted his infection through intimate contact with heavily infected typhoid secreta of rabbits and guinea-pigs. Most painstaking inquiries which were met by a liberal cooperation on the part of our janitor failed to reveal any possibility of outside connections with acute, latent or carrier typhoid fever cases. His whereabouts the last four months previous to his transitory illness were readily traceable on account of the illness of his wife and his compulsory functions as a nurse. The only determinable source was the infection from our experimental animals. It is well known to workers in this field of experimental pathology that renal (in the first) and gallbladder carrier rabbits (in the second place), as a rule, may shed enormous numbers of living, virulent typhoid bacilli. The sawdust bed of such carrier cages regularly contains demonstrable B. typhosus, and again the handling of typhoid animal cadavers is connected with even greater danger of exposure. Even with the average amount of care it is unavoidable that such material soil the hands of the cleaner or cremator. For this reason it has been our policy to protect our personnel by vaccination, repeated in from one to two years. Kisskalt,[38] in his recent summary of typhoid laboratory infections, mentions several cases which resulted from contact with animal material, particularly rabbit typhoid carriers.

[38] Ztschr. f. Hyg. u. Infektionskrankh., 1915, 80, p. 145.

One case is cited in which a laboratory janitor contracted typhoid fever through eating his meals in a stable where a goat had been inoculated with living typhoid bacilli. Thus it is apparently not an uncommon occurrence that such experimental material serves as a potent source for enteric fever infections. It may, however, be stated that undoubtedly some as yet unknown factors must be concerned, because the writers have in the last four years repeatedly aspirated or otherwise come in contact with heavily infected animal material, and neither of the two has contracted typhoid fever. It is our belief that the prophylactic vaccination practiced every 12 months is mainly responsible for the fortunate outcome of the unavoidable accidents.

Epidemiologically, case 2 is again explained on circumstantial evidence only. The patient is personally convinced that she contracted the infection in the course of her laboratory work in making innumerable slide agglutination tests. It is difficult, however, to state the date on which she probably became infected and particularly in the light of the interesting bacteriologic findings, it would be invaluable to know whether she infected herself with strain "I 75" isolated from case 1, or with one of the many typhoid strains in use at the time theoretically accountable for her illness. One thing is certain, that the identical organisms were tested by her which are considered responsible for case 1. An infection outside of the laboratory has been completely ruled out by a searching study of a possible source. Her first clinical symptoms developed about 50 days after she had handled strain "I 75," according to her records. There are two possibilities which may help to explain the identity of the strain "Chr. 76" isolated from case 2 with strain "I 75," either in the vaccinated person strain "I 75" remained latent or one or several of the experimental strains of B. typhosus were in her tissue transformed in a similar manner into an atypical strain as we assume it to be case for strain "I 75." Personally, we believe that the worker handled the culture "I 75" perhaps unknown to herself in a period shortly before she developed the clinical signs of typhoid fever. Repeated attempts to prove experimentally or otherwise the suggested explanation have failed and the identity of culture "I 75" and "Chr. 76" is the only tangible link which establishes the connection of case 2 with case 1.

The practical epidemiologist has no difficulty in explaining case 3. The handling of a spatulum used for the preparation in a stool specimen derived from a clinical typical typhoid fever case furnishes the connecting bridge between cases 2 and 3, and again, the daily presence

of a physician in the house of patient 2 establishes beyond any doubt the incubation time of 12 days for case 3; this fact would therefore be additional proof of the correctness of the deductions above stated. Such conclusions, however, appear doubtful to the bacteriologist when he notices striking cultural and serologic differences in the offending organisms of cases 2 and 3, and it is only natural to suspect that case 3 was the result of an outside instead of an inside house contact infection. One of us (N. M. N.) reviewed epidemiologically in detail the various typhoid cases which were known to exist in the community, and which were thought to be reasonable sources of infection. It was not only possible to prove conclusively that the infection did not originate from any one of the outside cases, but the members of the household also unanimously agreed that Sch. had not taken any meals outside of his house during the illness of his daughter. While the bacteriologic findings suggest a new source of infection, all the epidemiologic data prove conclusively a contact infection. The importance of this fact will become apparent in connection with the discussion of the bacteriologic findings.

2. *Clinical Data.*—The clinical findings in case 1 were indefinite, and without a positive blood culture an early accurate diagnosis would have been impossible. Also in case 2 the course of the first febrile attack was mild and clinical diagnosis was only ventured during the relapse. It is not unlikely that this infection would have ended abortively had the patient remained in bed a few days longer. In neither case were rose spots observed, nor was the spleen definitely palpable. The Widal reactions were negative, and even in applying Dreyer's principle, exceedingly doubtful. Even the blood culture method, which, according to recent accounts of Eggerth,[39] is the most reliable procedure for the diagnosis of typhoid fever infections in the vaccinated, failed completely. A typical leukopenia and a slow pulse were, however, suggestive of such a disease. Stool and urine examinations of case 1 were negative. In case 2 one urine specimen contained irregular typhoid bacilli. Negative stool specimens were to be expected on account of the carbohydrate diet, which as Torrey[40] and we have repeatedly shown, reduces the viable B. typhosus to such a degree that a bacteriologic demonstration is frequently impossible even with brilliant green eosin plates.

[39] Jour. Infect. Dis., 1919, 25, p. 166.
[40] Ibid., 1915, 16, p. 72.

These observations confirm the facts that have been established by the medical service of the U. S. Army and recently presented by Soper [41] at the meeting of the American Public Health Association, namely, typhoid fever in the vaccinated may as a rule run a mild course and one difficult to recognize. Clinically, it is also impossible to differentiate a paratyphoid from a typhoid infection. It therefore could be suggested by our critics that in the light of the serologic tests the infections were caused by a nongas-producing B. enteritidis or B. paratyphosus. The clinical observations lend little support to this contention. The B. enteritidis infections thus far reported by Jochmann [42] and observed by one of us, are always abrupt and in their initial symptoms governed by marked gastro-intestinal reactions. Furthermore, relapses as Torrens and Whitington [43] and Jochmann have pointed out, are comparatively rare and usually of shorter duration than in true typhoid fever.

It may be a mere coincidence when Kisskalt [37] states in his summary that in laboratory infections the Widal reaction is frequently negative. Concerning this point accurate data determined by the macroscopic agglutination test should be collected from future cases.

The diagnosis of typhoid fever in the vaccinated was apparently connected with difficulties in the Army, otherwise a classification into (1) suspected (2) clinically and (3) bacteriologically proved cases would not have been advocated. Little comment is necessary when dealing with the clinical aspect of case 3; the symptoms and bacteriologic findings were typical in every respect.

3. *Bacteriologic Findings.*—Our bacteriologic findings with one cell cultures have established the following facts: Strain "I 75" and "Chr. 76" isolated from cases 1 and 2, respectively, behave as irregular, atypical typhoid bacilli. Strain 49 isolated from case 3 is a typical B. typhosus. The irregular strains exhibited the following variants: They are rapid dulcite, rhamnose and irregular arabinose fermenters; they are "blue typhoids" producing rapidly alkalies in bromcresol purple milk; one strain produced indol in the second generation; serologically, they are hyperagglutinable as living organisms and belong to a subgroup of group III of Hooker's typhoid classification. They are typhoid-inagglutinable in formalinized suspensions, but are as saprophytic strains agglutinated by B. enteritidis serums and by their

[41] Am. Jour. Pub. Health, 1920, 10, p. 301.
[42] Lehrbuch d. Infektionskrankheiten, Berlin, 1914, p. 85.
[43] Brit. Med. Jour., 1915, 2, p. 697.

own with immune serums. Typhoid immune rabbits are protected against the infection and intoxication by the irregular strain. These variants, with the exception of an acquired agglutinability for B. enteritidis serums, have remained constant in the course of at least 150 transplants on peptic digest agar. It is evident, that the irregular strains differed bacteriologically and serologically only in degree from the true B. typhosus. Inherent properties, like certain carbohydrate fermentations (rapid fermenters) and coreactions with B. enteritidis serums, are enhanced to a marked degree of activity, but there are no suggestions of true mutation, a conception which is well supported by the masterly analysis of the available facts in Eisenberg's [44] summary on "Bacterial mutation." This paper contains all the important references to atypical typhoid or paratyphoid strains, but we have been unable to identify our organisms with any one of the hitherto described irregular bacteria of the typhoid paratyphoid group. In many instances the method of identification has been so incomplete that it would be mere guess-work even to correlate the organisms recently described by Guerbet and Henry, Faroy, Lafforgue, Messerschmidt, Marotte, Fromme, Oette, Wagner, Goebel, Ohno, Wille, Broughton-Alcock [3] and others with our own.

Irregular fermentation reactions are frequently accompanied by similar variations in the serologic behavior. Inagglutinable and peculiarly receptive strains have been repeatedly described, but an analogue to our observation was not found in the references at our disposal. It is, however, emphasized that agglutinability is a variable characteristic (Henderson Smith [45]), and that it should be used with caution in the differentiation of closely allied variants of the typhoid-colon groups.

It would be interesting, indeed, to know how this intensification of the inherent properties was induced and how far the resultant variants influence the pathogenicity and epidemiology of the disease, and to what degree the occurrence of such variants influences our conception of the homogenicity of the B. typhosus group. Is it a mere coincidence that the progressively atypical and irregular strains occurred in two typhoid vaccinated persons and apparently the same strain reverted atavistically to a typical typhoid strain in an aged nonvaccinated man? We are not in a position to answer these questions, because test-tube experiments and innumerable rabbit and guinea-pig experiments have

[44] Ergebn. Immunitätsforschung, 1914, 1, p. 28.
[45] Trans. Fifteenth Int. Congress of Hyg. and Demogr., 1912, 2, p. 99.

not enabled us to accomplish this transformation. Laboratory animals are nonsusceptible to the B. typhosus, and even extended latency of this organism in the bile or tissues of rabbits, guinea-pigs, dogs or monkeys has in our experience created only inagglutinable but otherwise typical offsprings. A certain degree of adaptability to changes in the H-ion concentration of the substratum may be noted as was recently pointed out by one of us. But this fact has remained thus far the only tangible suggestion that existence in animal tissues may be conducive to the production of variants. The literature, however, contains sufficient observations which indicate that fermentative properties may be acquired on artificial mediums. B. typhosus can, as Twort,[46] Penfold,[16] R. Müller [15] and others have shown, gradually "mutate" into distinct variants, which, however are inconstant. After a series of cultivations on a substratum free from the enhancing carbohydrate fermentation stimulating substance, the variants as a rule revert to the original ancestral type.

It is quite plausible to assume that such a transformation of one or several true typhoid strains occurred in our case 1. In this connection we cannot overlook the possibility that perhaps animal paratyphoid strains to which the man was exposed underwent transformation. A change of their labile properties is in the realm of possibilities. Such a conception as well as the criticism that we were possibly working with mixed cultures can be readily dismissed. Old and recent observations have proved the low pathogenicity of animal strains of B. paratyphosus B and B. enteritidis, and again our discussion of the bacteriologic and serologic findings given above lend little support to this assumption. Repeated plating and finally the preparation of one cell cultures by the Burri method were chosen to rule out conclusively the danger of a mixed strain.

Passing through a second vaccinated host the irregular strain undoubtdly met an environment similar to the first, and it therefore preserved the new properties acquired. Only when flourishing in the tissues of an unvaccinated individual the variant reverted for reasons as yet unknown to the original typical B. typhosus. In our opinion, the most important question: "Is a change in metabolic activities also accompanied by alterations in pathogenic and antigenic properties?" cannot be answered conclusively. Animal infection and intoxication experiments fail to inform us concerning the true pathogenicity for

[46] Proc. Roy Soc., London, 1907, B. 79, p. 329.

man, and we are mainly dependent for an explanation on the results of our serologic tests. It was noted that strain "I 75" and "Chr. 76" differed in many respects from the type strain "Rawlings," and that these strains belong to a subgroup of the B. typhosus. It is the recognition of this fact that prompted this report and that furnishes some questions of considerable practical importance. The answers to some of these queries can perhaps be best introduced by the citation of a few lines from the recent book of Adami:[47]

> Still I shall feel that these pages have not been written in vain if I succeed in drawing increased attention to the fact that the bacteria are organisms acutely susceptible to changes in environment, that as species they are far from presenting constant characteristics, and that to a variability which may impress itself upon a greater or less number of generations is to be ascribed, in part, the differences between successive epidemics, between the successive stage of one epidemic, and between individual cases of disease.

We are well informed concerning the variability of the coccus group; but few authentic observations have thus far been made with micro-organisms of the typhoid group. This is in part the result of incomplete epidemiologic investigations and of abbreviated methods for the identification of the typhoid bacillus which will rarely disclose functional variants and therefore will never lead to the discovery of new types responsible for certain epidemics. As a matter of fact, the epidemiologist of today is frequently considered nothing more than an expert in detective and police methods, when his highest function primarily should consist of the intricate analysis of all the biologic and dynamic forces leading to an epidemic. For this reason he should have at his command a laboratory of his own, and a force of experts fully equipped to study biologically the suspected causes. With the progress of our methods of sanitation and preventive immunization the necessity for the detection of new disease producing variants should be proclaimed with unremitting insistence. Perhaps for no other disease is this demand more urgent than for typhoid fever. Only when these studies have been made on every occasion will it be possible to answer the mooted question: Does the prophylactic vaccination with a monovalent antigen really confer the maximum obtainable protection? It is not our intention to enter into a consideration of this controversy, but the observations of Mock,[2] Kisskalt[37] and our own clearly demonstrate that it is the vaccinated or the laboratory worker, who develops irregular typhoid strains. We should, therefore, insist on a most careful study of the bacterial strains from these sources.

[47] Medical Contributions to the Study of Evolution, New York, 1918, p. 131.

SUMMARY

This report describes the laboratory infections in a vaccinated caretaker (case 1) exposed to laboratory animals shedding living typhoid bacilli and a laboratory worker also immunized (case 2), who was regularly working with strains of the B. typhosus group. The last case apparently caused a severe and fatal house contact infection (case 3). From the blood of case 1 and the urine of case 2, on one occasion only, an irregular, atypical organism, and from the stool and urine of case 3 typical typhoid bacilli were isolated. The irregular typhoid strains ferment without gas production the usual carbohydrates, also dulcite, rhamnose and irregularly arabinose; they rapidly cause an alkaline reaction in milk, and develop small rhamnose papillae comparatively slowly. In formalinized killed suspensions they are only agglutinated by their own immune serums and recently also by B. enteritidis serum. As living organisms they are specifically clumped and sedimented by typhoid immune serums and can be classified by absorption tests with one of the subgroup of group III in Hooker's classification. Antiserums prepared with these organisms agglutinate typhoid bacilli in living suspension only and coagglutinate slightly, if at all, the representatives of the paratyphoid B or A group. As saprophytic strains they behave like nongas-producing strains of B. enteritidis from which group they cannot be separated even by careful absorption tests.

The possible bearing of these observations on the epidemiology, clinical aspect and bacteriology of typhoid fever is discussed, and it is suggested that special attention be paid to the occurrence and the detailed study of irregular typhoid strains in the typhoid-vaccinated.